Talanta 116 (2013) 559–562

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Development of a fast capillary electrophoresis-time-of-flight mass spectrometry method for the speciation of organotin compounds under separation conditions of high electrical field strengths

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ARTICLE INFO

Article history:
Received 18 April 2013
Received in revised form
10 July 2013
Accepted 11 July 2013
Available online 21 July 2013

Keywords:
Capillary electrophoresis
Time-of-flight mass spectrometry
Dibutyltin
Tributyltin
Diphenyltin
Triphenyltin
Speciation analysis
Water samples

ABSTRACT

A novel approach has been developed for the separation of organotin species with capillary electrophoresis hyphenated to time-of-flight mass spectrometry. It has been applied to the development of a method for the determination and speciation of organotin compounds namely, dibutyltin (DBT), tributyltin (TBT), diphenyltin (DPT) and triphenyltin (TPT) in water samples. Experiments were made with a special laboratory constructed CE instrument. A non-aqueous buffer system compatible with TOF-MS has been developed using ammonium acetate–acetic acid (50 mM and 1 M) in acetonitrile: methanol (80:20). The total analysis time is less than 3 min for these compounds under the conditions developed. The method has been applied successfully to the determination of these compounds in river water samples. Detection limits of the CE-TOF-MS method were between 1 and 8×10^{-7} M, and between 2 and 11×10^{-9} M (0.46 to 3.2 $\mu g\,L^{-1}$) when used in conjunction with solid phase extraction. The short analysis time as well as good sensitivity and selectivity make it a useful approach for the fast screening of organotin compounds.

1. Introduction

Many organotin compounds are important organometallic pollutants in the aqueous environment [1]. Oganotin compounds find wide application in various areas. Tributyltin (TBT) and triphenyltin (TPT) are commonly used as anti-fouling agents in paints [2]. These compounds also have important industrial applications like in agriculture as pesticides and as wood preservatives and as PVC stabilizers. Due to their biocidal properties in agriculture these have toxic properties associated with them. Important organotin compound is tributyltin (TBT) which is an active ingredient in antifouling paints. As a result, most countries have banned the use of antifouling paints containing tributyltin (TBT) on small boats, and it was included in the European Union priority pollutant list for fresh waters: RD 995/2000 defines TBT as a quality parameter and establishes its annual medium value as 0.02 μg/L. The pollution by triphenyltin (TPT) which is considered as a non-selective pesticide, also poses a serious problem as it accumulates in lipophilic tissues.

Recently it has been considered by the European Union [2] that TBT must be included in the list of compounds which require regular monitoring of fresh waters. Consequently, there is a strong need for the development of advanced methods for the determination of organotin compounds that provide sufficient sensitivity, selectivity and accuracy. Due to different species of organotin present in the environment the speciation analysis of these compounds is very important in environmental samples [3]. Generally, the determination of organotin compounds is carried out by a separation technique in combination with a suitable detector system. One approach to achieve relatively low detection limits is gas chromatography coupled to mass spectrometry. But this technique suffers from the drawback that it requires derivatisation of the compounds which is tedious and time consuming. Various other methodical approaches involving GC-ICP-MS or GC-ICP-AES, fluorimetry and liquid chromatography [4–9] have been described. The latter technique, liquid chromatography, offers various advantages like robustness and high sensitivity when combined with detectors such as MS, ICP-MS or ICP-AES and meets the requirement of the detection limit and sensitivity of these compounds in the environment. The major drawback of this method is the irreversible interaction of the organotin compounds with the stationary phases of the columns, which causes considerable band broadening and other serious problems.

Abbreviations: TBT, Tributyltin; DBT, Dibutyltin; TPT, Triphenyltin; DPT, Diphenyltin

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http://dx.doi.org/10.1016/j.talanta.2013.07.025

Capillary electrophoresis offers various advantages such as high separation efficiency, shorter analysis time and low solvent consumption. Various CE-UV methods for the determination of these organotin compounds were reported in the literature [10–14], but these suffer from various disadvantages like sensitivity and selectivity. As most organotin compounds show poor UV absorption the sensitivity of CE-UV methods is a critical aspect [14]. Indirect UV detection [10,11] can be applied but is usually associated with limited robustness. The combination of CE with more sensitive and selective detectors, such as mass spectrometers can considerably enhance the analytical performance. During the recent years, there is a considerable demand for an increased speed of analysis and it has lead to the development of various methods and techniques for analysis. A conventional CE system uses long capillaries (75 cm) so that analysis times are quite long. Recently, a new approach was introduced by our group [15–17] using short capillaries coupled with time-of-flight-mass spectrometry (TOF-MS). Field strengths exceeding 1 kV cm^{-1} have been applied for separations of model analytes resulted in migration times on the time scale of seconds. The attractive characteristics of TOF-MS in the context of complex samples and fast CE are high mass accuracy and fast acquisition rates.

To our knowledge, this is the first time that CE-TOF-MS has been applied to determine and speciate organotin compounds using short capillaries under high electric field strengths. The application to the analysis of water samples is demonstrated.

2. Materials and methods

2.1. Chemicals and solutions

DBT chloride, TBT benzoate, TPT chloride, DPT chloride and acetic acid (HAc, 99.9%) were all purchased from Aldrich (Milwaukee, WI, USA). Ammonium acetate, methanol (MeOH) and acetonitrile (AN) were obtained from Merck (Darmstadt, Germany). All reagents were of analytical reagent grade or better. Standard solutions of organotin compounds in the concentration range of 5–1000 μM were prepared in HPLC-grade AN and kept at 4 °C in the dark. For CE injection, the organotin samples were freshly prepared by dilution with appropriate background electrolyte (BGE). CE BGE were prepared by mixing appropriate amounts of HAc, NH_4Ac, MeOH, AN. A solution containing 50 mM ammonium acetate, 1 M acetic acid in acetonitrile/methanol (80:20, v/v) was used as BGE for all experiments reported here. Before use, the BGE solutions were filtered through a 0.45 μm syringe filter and degassed by sonication for 4 min.

Phenomenex (Torrance, CA, USA) strata C18-E 500 mg SPE cartridges were used for sample extraction.

A solution of 2-propanol/water (50:50, v/v) containing 0.3 mM formic acid and 10 mM sodium hydroxide was used to create sodium formate clusters for mass calibration as well as for tuning the TOF-MS for highest sensitivity in the desired mass range.

2.2. Instruments

A micrOTOF-MS (Bruker Daltonik, Bremen, Germany) was used in conjunction with a home-made CE system consisting of a high-voltage power supply (Model HCN 7E-35000, F.u.G. Elektronik, Rosenheim-Langenpfunzen, Germany) and a manual sample injection system. The CE-TOF-MS coupling was done by means of a coaxial sheath-liquid sprayer (Agilent Technologies, Waldbronn, Germany) using isopropanol/water (50:50 v/v) containing 0.2% formic acid as sheath liquid. Electrical contact at the electrospray needle was established via the sheath liquid delivered at a flow rate of 8 μL min^{-1}. Fused silica capillaries (50 μm ID, 360 μm OD) of 75, 65, and 50 cm length were used. The separation capillary was kept at 15 °C during separations.

2.3. Sample collection and extraction

Water samples were collected in PE bottles from the Danube River in Regensburg, Germany.

Aliquots of 450 mL were each spiked with 267 μL of a stock solution containing 3×10^{-5} M of each analyte, resulting in a spiked concentration of 1.8×10^{-8} M. Blank samples and spiked samples were then subjected to the following procedure.

The sample solution was acidified to pH 2 with dilute HCl and filtered through a 45 μm membrane filter. SPE cartridges were conditioned with 5 mL of methanol followed by 10 mL of acidified Millipore water (pH=2). The sample solution was then passed through the cartridge at a rate of 5 mL/min. The cartridge was rinsed with 10 mL water and dried by pumping air through it for 2 min. Elution of the analytes was performed with 4 mL of CE buffer and the volume of the extract was then adjusted to exactly 4.00 mL.

3. Results and discussion

3.1. Optimization of TOF-parameters

Method development for TOF experiments consisted of two parts. Firstly, the instrument was tuned to work with highest sensitivity in the mass range of 250–700 m/z where any species of the compounds studied were expected. Secondly, individual standard solutions were injected without applying any separation voltage to the capillary. The signal clusters with typical tin isotope pattern were evaluated as to whether they can be attributed to any possible species (monomer or dimer, with different adducts). An additional set of mass spectra was then recorded under separation conditions for each compound in order to rule out any solvent-dependant adduct formation due to the different solvent composition in analyte stock solution and CE buffer. Calculated and recorded mass spectra for species derived from DPT and TPT are shown in Fig. 1. While both DPT and DBT showed two species with different adducts each (formate (sheath liquid component) and acetate (background electrolyte component)), TPT and TBT exhibit a tendency to form complex dimers in addition to simple molecular ions. Only singly charged ions were observed. Calculations of mass spectra were carried out using the *Simulate Pattern* feature of the Compass Data Analysis software using the molecular formula of the species shown. The good agreement between theoretical and experimental mass values allowed for the use of very narrow mass windows for calculation of the extracted ion traces, effectively lowering the *S*/*N* ratio.

Due to tin's complex isotope pattern, selection of m/z-signals for the calculation of extracted ion traces requires some thought as to the signal-to-noise ratio obtained. While it would be possible to include all major isotope signals (intensity greater than 5% of the most abundant isotope, hence 7 signals for monomers and 13 for dimers), there would not only be an increase in sensitivity, but also in the baseline noise of the corresponding trace. For this reason, only those five individual m/z-signals with the highest intensity of a given species where used to calculate extracted ion traces, totaling 10 signals per trace.

3.2. CE-TOF-MS method development

In CE-TOF-MS the sheath-liquid interface induces a suction pressure which results in an additional flow contribution. Compared to CE-UV, where no such pressure exists, separation times in CE-MS for a given specification of capillary length, capillary ID and high voltage are considerably shorter.

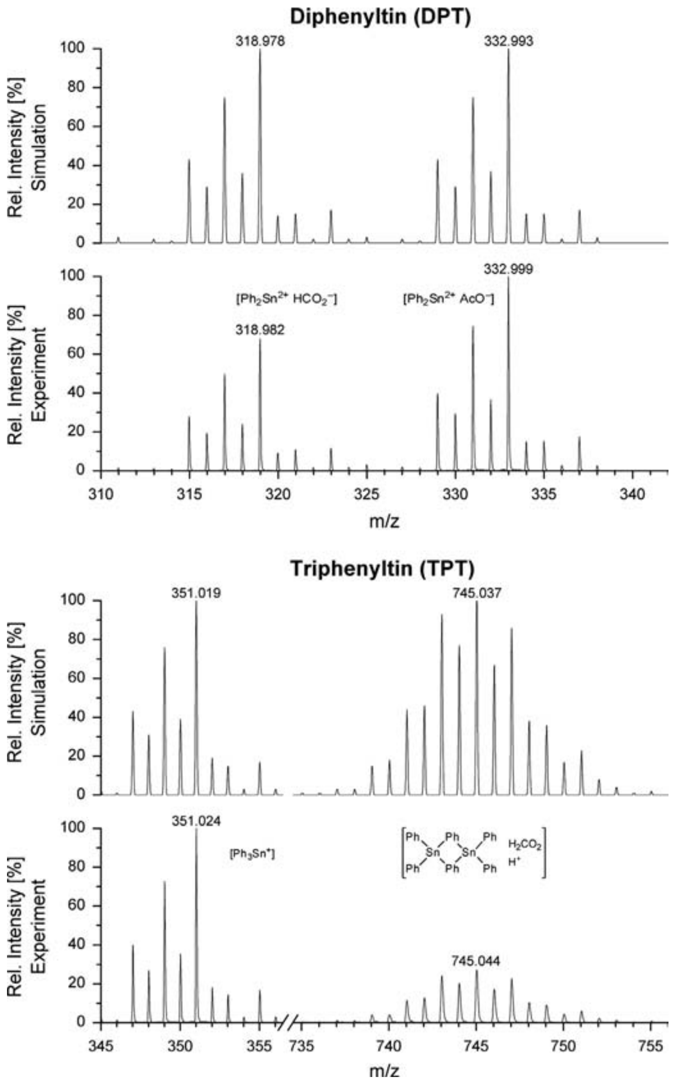

Fig. 1. Calculated (upper) and recorded (lower) mass spectra for typical species derived from DPT and TPT. The proposed structures for the species found as well as the exact masses of the base peak of each species are given. The analytes were dissolved in a solution containing 50 mM ammonium acetate, 1 M acetic acid in acetonitrile/methanol (80:20 v/v).

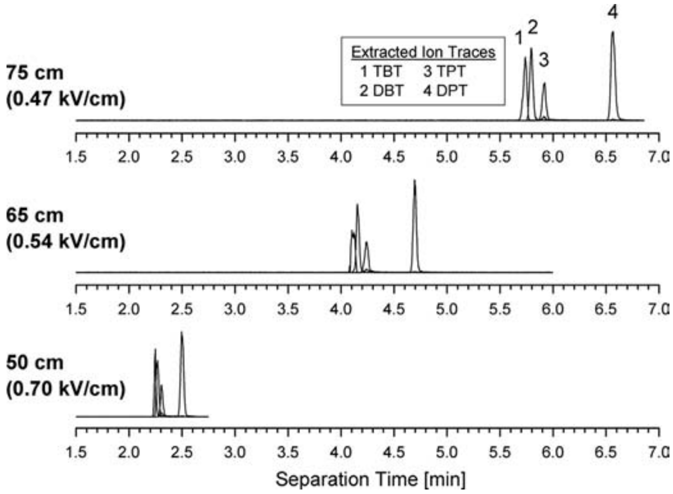

Fig. 2. CE–TOF-MS separations of organotin compounds using capillaries with different lengths. A background electrolyte as specified in Fig. 1 was used, the separation voltage was 35 kV.

Injection of sample solution was carried out hydrodynamically by transferring the capillary from the buffer vial to the sample vial for a defined injection period. After determining the linear flow through the capillary in a flow-injection experiment, the injection time was chosen to correspond to an injection plug of around 1.5% of the capillary length. This value was found to be the optimum regarding signal intensity and peak broadening, with longer injection times leading to increased band broadening while not considerably gaining signal intensity. For a capillary of 50 cm length, this corresponded to an injection time of 5 s.

Owing to the simpler setup, shorter capillaries can be used with the laboratory-made CE-system. Fig. 2 shows the effect of decreasing the capillary length from the initial 75 cm to 65 and 50 cm, while working at 35 kV. As can be expected, both total separation time and resolution decreased. The shorter analysis times of only 2.5 min at 50 cm, however, was seen as more valuable than achieving baseline separation. In particular, fast CE-TOF-MS determinations are attractive in the context of fast screening of organotin species.

In another set of experiments, the influence of high voltage field strength (0.4 to 0.7 kV cm^{-1}) was evaluated, working with a capillary length of 50 cm. It was found that the decrease of resolution with increasing field strength is negligible compared to the shorter analysis times. A capillary length of 50 cm and a separation voltage of 35 kV (0.7 kV cm^{-1}) were chosen for all further experiments.

3.3. CE-TOF-MS method evaluation

Calibration curves for the four analytes were recorded using the final CE-TOF-MS method. They were found to be linear over the concentration range investigated (3×10^{-7}–3×10^{-5} M). Table 1 details the analytical parameters. The differences in sensitivity for the four compounds studied can be attributed to different ionization efficiencies on the one hand, and to different noise intensities of the extracted ion traces on the other hand. The limits of detection are about two orders of magnitude lower than those obtained using UV detection [14]. Deviations in migration time always affected all analytes in the same way; no change in the order of migration was observed and the effect on signal resolution was minimal.

3.4. Application to river water samples

River water samples were collected and subjected to the extraction procedure detailed in section 2.3. Fig. 3 shows electropherograms of extracts from blank and spiked samples. The blank samples showed no detectable signal for any of the analytes under investigation. No other compounds with tin isotope pattern could be found in the extracts. The main drawback of the CE-UV analysis is that the samples show a number of signals with migrations times in the range of the analytes [14]. Inspection of the corresponding migration intervals in the CE-TOF-MS analysis, however, clearly proved that these signals have to be assigned to matrix components, emphasizing the advantages of mass spectrometric detection.

Table 2 summarizes the analytical characteristic of the extraction protocol. Recoveries vary for the different organotin compounds, with tri-substituted species showing better retention than di-substituted ones as well as phenyl-substituted analytes showing better retention than butyl-substituted ones. With an SPE preconcentration factor of 113 and recoveries between 44 and 77%, effective preconcentration factors between 49 and 87 were achieved.

4. Conclusions

An analytical method for the fast determination of four organotin compounds was developed by hyphenating capillary electrophoresis

Table 1
Analytical characteristics of the CE–TOF-MS method for the determination of organotin compounds.

	TBT	DBT	TPT	DPT
Calibration range (10^{-7} M)	10–300	10–300	3–300	3–300
Regression coefficient	0.998	0.99994	0.999	0.9997
Limit of detection ($S/N=3$) (10^{-7} M)	8.1	3.9	2.4	0.98
RSD of peak height ($n=3$) (3×10^{-5} M) (%)	12	6	14	8
Migration time (min)	2.24	2.27	2.31	2.5
RSD of migration time ($n=3$) (%)	3.9	4	4	4.7

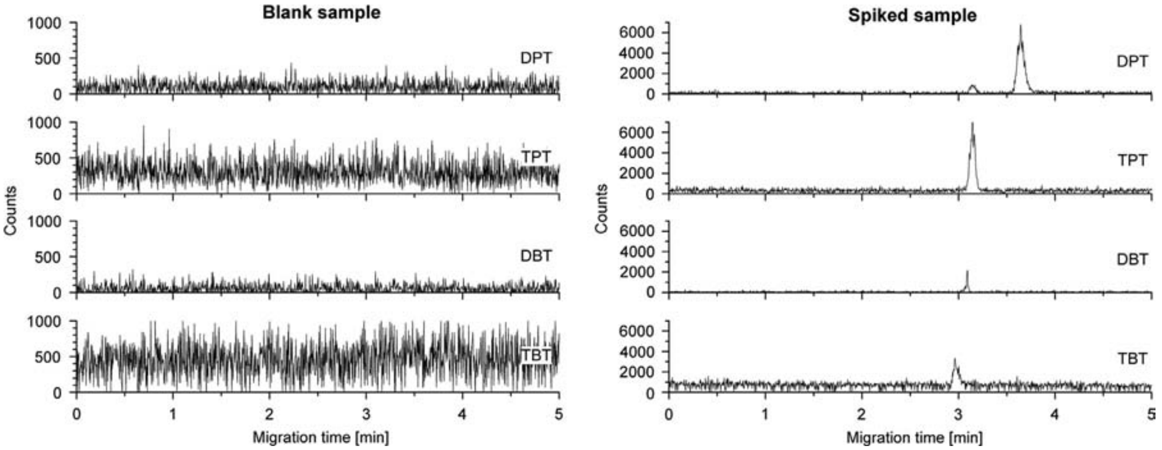

Fig. 3. CE–TOF-MS separations of extracts obtained from blank and spiked river water samples. Spiked concentrations were 1.8×10^{-8} M. The graphs show the different extracted ion traces singly for clarity while using the same scale on the y-axes for each separation for better comparison of the different S/N characteristics.

Table 2
Analytical characteristics of CE–TOF-MS with SPE extraction for the determination of organotin compounds. The protocol for sample extraction is specified in section 2.3.

		TBT	DBT	TPT	DPT
Recovery (%)		66.0	43.9	77.4	52.1
RSD of recovery ($n=3$) (%)		5.7	16	2.5	7.3
Effective pre-concentration factor		74	49	87	59
Limit of detection with pre-concentration with CE–TOF-MS method ($S/N=3$)	(10^{-9} M)	11	7.9	2.7	1.7
	(μg L^{-1})	3.2	1.8	0.94	0.46

and time-of-flight mass spectrometry. It only requires minimal sample preparation, has a low solvent consumption and separations can be achieved in less than 3 min. The LODs of the method are between 1 and 8×10^{-7} M ($S/N=3$).

In addition, a solid phase extraction method was developed for the determination of the four analytes in water samples. Using spiked river water, recoveries from 44% to 77% with relative standard deviations between 2.5% and 16% could be obtained. LODs of the overall protocol were found to be between 1.7 and 11×10^{-9} M (0.46 to 3.2 μg L^{-1}).

Using time-of-flight mass spectrometry as detection method proved particularly useful when analyzing real samples since it allowed for the unambiguous differentiation between analytes and matrix components, a common problem when using a non-specific detection method like UV/VIS [14].

Acknowledgment

AKM is thankful to the Alexander von Humboldt Foundation (Bonn, Germany) for the research fellowship during his stay in Germany.

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